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Mini-Review

A temperature-sensitive TRP ion channel, Painless, functions as a noxious heat sensor in fruit flies

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Abbreviations: TRP, transient receptor potential; HEK293 cells, human embryonic kidney 293 cells

Key words: TRP channel, temperature sensation, noxious heat, drosophila, Ca^{2+} ion

Animals must be capable of sensing hazardous temperatures to avoid physical injury. Recent progress has revealed the molecular mechanisms underlying this capability. This essential function requires a subset of the Transient Receptor Potential (TRP) channel family in both mammals and Drosophila. We recently showed that a Drosophila TRP channel, dubbed Painless, possesses heat sensitivity that is essential for avoidance of noxious heat. The temperature threshold for Painless activation is consistent with the temperatures that cause avoidance behaviors in vivo, indicating that Painless acts as a primary noxious heat detector in Drosophila. In this review, we summarize the properties of temperature-sensitive TRP channels, including Painless, in fruit flies.

Temperature-Sensitive TRP Channels are Physiological Thermosensors

The ability to sense environmental temperature is necessary to discriminate between permissive habitats and hazardous environments that cause injury. Recently, it has been revealed that several members of the TRP ion channel super family are activated by temperature changes. The temperature sensitivity of molecules is usually evaluated with their Q_{10} values, which are defined as ratios of reactions at 10-degree increments. Many enzymes and channels have Q_{10} values less than 3,[1] whereas the thermosensitive TRP channels (thermoTRPs) have extremely high values greater than 10. A number of reports clearly demonstrate that thermal activation of these TRP channels contributes to various temperature-dependent responses in vivo, such as thermosensation, thermotaxis and regulation of cellular/tissue functions at physiological body temperature. So far, nine TRP channels have been reported to respond to a physiological range of temperatures in mammals.[2] TRPV1 and TRPV2 expressed in nociceptive neurons are activated by heat (>43°C and >52°C, respectively), and TRPV1-null mice have defects in sensing noxious heat.[3] TRPV3 and TRPV4 are predominantly expressed in skin keratinocytes rather than in sensory neurons, and gene knock-out of each channel causes abnormal thermotaxis in vivo.[4,5] TRPM8, which senses cold temperatures (<27°C),[6,7] is expressed in nociceptive and non-nociceptive neurons and its loss impairs cold sensitivity.[8-12] TRPA1 is expressed in nociceptive neurons[13] and acts as a sensor for various harmful stimuli, whereas its responsiveness to noxious cold stimuli is controversial even after the analysis of mice lacking the channel.[14-17] Other thermoTRPs, TRPM2, TRPM4 and TRPM5 are not expressed in sensory neurons, and are reportedly involved in several functions at physiological body temperatures including insulin secretion, taste sensation and immune response.[18-20] Thus, the thermoTRPs are now considered to be primary elements for temperature-dependent responses.

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TRP Channel-Dependent Thermosensation in Fruit Flies

In fruit flies, some TRP channels are important for temperature detection like in mammals. In *Drosophila melanogaster*, thirteen genes encode TRP channels. Five of those channels belong to the TRPA and the TRPC subfamilies, which contribute to temperature-related behaviors (Fig. 1). dTRPA1 was first identified as a heat-activated channel (>27°C) in vitro,[21] and its knockdown by RNAi[22] or gene-knockout[23] revealed the importance of the channel for thermotaxis from warm temperature to a preferred range (18–24°C). dTRPA1 proteins are expressed in anterior cell neurons, two pairs of neurons at the brain's anterior.[24] Pyrexia is a noxious heat-sensitive TRPA channel with high potassium permeability, and it may protect flies from high temperature stress.[25] Pyrexia-expressing sensory neurons are widely distributed throughout the body. Another TRPA subtype, dubbed Painless, was found to be essential for avoidance of noxious heat above 40°C in Drosophila larvae and adults.[26,27] The protein was found at the tips of the dendritic arbor in a small subset of multidendritic sensory neurons which resemble our peripheral afferent neurons. More recently, TRP and TRPL (belonging to the TRPC subfamily), which are critical for vision in Drosophila, were reported to be involved in cold avoidance.[28] This evidence strongly demonstrates that certain TRP channels share a common role in

temperature sensation between distantly related species. Interestingly, in flies, four channels are classified in the TRPA subfamily and three of them are known to contribute to thermosensation. Therefore, the TRPA subfamily may play a central role in temperature detection and related behaviors in insects.

Noxious Heat Sensitivity of Painless

Drosophila melanogaster prefer temperatures in the range of 18–24°C. They quickly escape from noxious temperatures above 40°C. Wild-type larvae display stereotypical 'rolling behavior', which is defined as a vigorous rolling movement lateral to the body axis, within one second of being touched with a heated probe.[26] Genetic screening to obtain mutants that have abnormal response against noxious heat identified *painless* mutants. These mutant larvae required much longer times to escape from a heated probe, indicating that the *painless* gene is essential for heat avoidance. The mRNA and the protein appeared to be localized in the tips of multidendritic sensory neurons of the peripheral nervous system, which resemble peripheral sensory neurons in mammals. The firing of these neurons increased upon heating over 38°C in wild type neurons, whereas such increases were not seen in the *painless* mutant. The Painless protein is predicted to have ankyrin repeats in the N-terminal region, and six transmembrane domains with one putative pore-forming region between transmembrane domains 5 and 6, all of which are common structures for the TRP ion channel super family. Since two Drosophila TRPA channels, dTRPA1 and Pyrexia, have been shown to have temperature sensitivity both in vitro and in vivo, it has been inferred that Painless might also be a heat-sensitive TRP channel. Indeed, we first revealed that the channel actually functions as a thermosensor.[29] Painless expressed in human embryonic kidney (HEK) 293 cells showed transient inward currents upon heating at negative membrane potentials, and the temperature thresholds for activation (42–44°C) were consistent with the temperatures that cause avoidance behavior in vivo.[26] Painless displayed heat responses in a cell-free excised membrane patch, indicating that Painless may sense heat directly, without utilizing intracellular signaling molecules. These in vitro analyses clearly demonstrate that in Drosophila, Painless itself is a primary heat detector, the activation of which raises neural activity against dangerous heat.

Functional Regulation of Painless by Ca^{2+}

A unique property of Painless is the extremely high and selective permeability of Ca^{2+}, which is almost 42 times higher than that of sodium.[29] Intriguingly, the various activation properties of the channel are affected by Ca^{2+}, all of which seem to be correlated with the flies' physiology. This interpretation is supported by the following observations. First, Ca^{2+} enables Painless to be heat-sensitive. Painless failed to respond to heat in the absence of intracellular and extracellular Ca^{2+}, whereas 200 nM intracellular Ca^{2+} restored the heat-dependent activation. The mammalian thermoTRPs such as TRPM4, TRPM5 and TRPA1 are activated by intracellular Ca^{2+},[18,30-32] whereas Painless requires Ca^{2+} as a co-agonist for heat-evoked activation. A similar concept has been reported in TRPM8, in that intracellular Ca^{2+} supports robust icilin-evoked responses.[33] Intracellular Ca^{2+} (100–200 nM) was sufficient for Painless activation, a concentration close to the reported value in the terminal and dorsal organs of the larval head.[34] Second, Ca^{2+} accelerates Painless

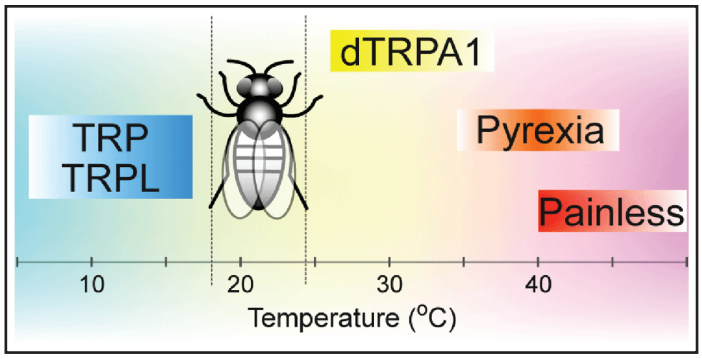

Figure 1. TRP channels and thermosensation in Drosophila. Flies utilize TRP channels to sense environmental temperatures. dTRPA1 is activated above 27°C and contributes to avoidance of warmer temperatures. Pyrexia is activated around 40°C and has a higher potassium permeability. Channel activation prevents paralysis during high temperature stress. Painless is activated above 40°C, and is essential for avoiding hazardous temperatures. These channels belong to the TRPA subfamily and posses temperature sensitivity. TRP and TRPL channels, both of which belong to the TRPC subfamily, are involved in cool temperature avoidance. However, the temperature sensitivity of these channels is unknown. The preferred range of *Drosophila melanogaster* (18–24°C) is indicated by dotted lines.

activation. Channel activation is rapid in the presence (but not in the absence) of physiological levels of intracellular Ca^{2+}, which reflects the fly larva's rapid rolling response when challenged by a heated probe.[26] Third, Ca^{2+} sensitizes Painless to heat. The temperature threshold for activation was ~42.6°C in the presence of intracellular Ca^{2+} regardless of the presence of extracellular Ca^{2+}, which was significantly lower than that in the presence of extracellular Ca^{2+} alone (~44.1°C). Quick avoidance from a heated probe occurred around 42°C in fly larvae.[26] Therefore, the in vivo temperature threshold seems close to that obtained in the presence of intracellular Ca^{2+}. Fourth, Ca^{2+} sensitizes Painless upon repetitive heating. Significant reduction in the temperature thresholds by more than 1°C was observed in the presence of extracellular and intracellular Ca^{2+}, but not in the presence of intracellular Ca^{2+} alone upon repeated heating, suggesting that extracellular Ca^{2+} and/or Ca^{2+} influx play a role in the sensitization. This is reasonable because flies are able to escape from a hazardous heat source in less time after a second exposure. Sensitization upon repetitive heating is a common feature in TRPV1, TRPV2 and TRPV3,[35,36] although the underlying mechanism is still unknown, including the requirement for Ca^{2+}.

Ca^{2+}-dependent regulation is partly ascribed to the properties of the N-terminal region of Painless.[29] Specific amino acid substitution (N363A), which is located in the ankyrin repeat domain, displayed small heat-evoked currents, increased temperature thresholds, and higher intracellular Ca^{2+} requirement with a reduced Hill coefficient. These results suggest that N363 is a key residue in Ca^{2+} sensitivity of Painless. Interestingly, the amino acid sequence, which includes N363, has partial similarity to the EF-hand-like domain in mammalian TRPA1. The EF-hand-like motif is reported to be responsible for Ca^{2+}-evoked activation of human TRPA1 which was impaired by an amino acid substitution.[31,32] However, the function of a Ca^{2+}-regulatory site in Painless seems different from that of the EF-hand-like motif. TRPA1 requires Ca^{2+} at a micromolar range for its activation, whereas Painless was not activated by intracellular Ca^{2+}

alone and requires much less Ca^{2+} for its modulation. Furthermore, mutation of N356, S357 or D366 did not affect the properties of Painless, while the corresponding amino acids in the EF-hand-like motif are critical for Ca^{2+}-dependent TRPA1 activation. Such Ca^{2+}-dependency upon thermal activation is unique to Painless.

Is Painless a Polymodal Receptor?

Our investigation demonstrated that Painless did not respond to typical stimuli known to activate mammalian thermoTRPs.[29] This is somewhat surprising because most mammalian thermoTRPs are activated by various stimuli in addition to temperature,[37-39] and Painless has also been reported to be involved in sensitivity to allyl isothiocyanate, a wasabi ingredient, sugar or mechanical stimuli in vivo.[26,40,41] Expression of *painless* was seen in a subset of sensory neurons among gustatory bristles located in the labial palpus, the legs, the wings, and the internal pharyngeal sensory structures in adults, all of which have been known to contribute to gustatory detection.[40] Expression of *painless* was also observed in mechanically-sensitive Johnston's organ and the central nervous system including the mushroom body,[26] where Painless could be activated by mechanical stimuli or endogenous ligands, respectively. Therefore, Painless possibly senses multiple stimuli other than heat, which might require endogenous accessory proteins, heteromeric channel formation, splice variants and/or phosphorylation. GPCR signaling might also regulate Painless function since the G_q-PLC pathway has been implicated in modulation of dTRPA1-dependent thermosensation.[23] These observations must be confirmed under more physiological conditions. It should be noted that heat activation of Painless was inhibited by ruthenium red and camphor,[29] which was reminiscent of the properties of mammalian TRPA1. This feature suggests some common mechanisms for channel activation within mammalian and Drosophila TRPA channels.

Thus far, no chemical or other physical stimuli have been available for activation of invertebrate thermoTRPs. Identification of new activators would be useful to control insects' behavior as the TRP channels primarily contribute to sensing mechanisms in invertebrates as well as in vertebrates. Camphor, a wood derivative from camphor laurel, has been used as a repellent for pests and proved to be effective for mosquitoes.[42] It would, therefore be intriguing to test the effects of camphor at the behavioral level, in spite of its inhibitory effect on Painless. Since activation of Painless evokes 'avoidance' in larvae and adult flies,[26,27] this channel is a good target for the development of new insect repellents.

Future Tasks: Analyzing the Mechanism of Thermosensation

Identification of the thermoTRPs sheds light on molecular mechanisms for temperature sensation. However, compared to the recent remarkable progress in mammalian research fields, understanding of the molecular properties and the physiological roles of thermoTRPs in ectotherms and invertebrates is quite limited, although the temperature sensation must be more important for them than for endotherms. Recent studies, including our own, provide clear evidence that fruit flies utilize specific channels to detect warm to hot temperatures, which emphasizes the significance of the evolutionarily conserved property of the TRP channels. In contrast, little is known about the mechanisms for cold sensation in Drosophila. They avoid undesirable cold temperatures (<18°C) and some specific neurons appear to respond to cold exposure.[34] Therefore, Drosophila must have specific cold sensor(s) like TRPM8 in mammals. In addition to TRP and TRPL channels,[28] it has been reported that histamine signaling molecules including a histamine-gated chloride channel, and a cyclic AMP-PKA pathway are involved in temperature preference and cold tolerance in Drosophila.[43,44] Furthermore, a recent report demonstrates that TRPA1 modulation via a G_q-PLC pathway participates in discriminating cooler temperatures in an optimal range (18–24°C).[23]

We have clarified the activation properties of Painless, and they are better understood than any other insect channel. Given that TRP channels play a primary role in sensation, elucidation of the channel properties in various insects would be beneficial to our understanding and facilitate control of their physiological responses. Genetic and behavioral analyses could reveal the basic mechanisms for temperature sensation in fruit flies, which may help us understand the complicated thermoregulatory system in mammals. Comparison of the function of the thermoTRPs among various species may also help clarify how specific channels are activated by temperature changes.

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